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Viral vectors for neurotrophic factor delivery: A gene therapy approach for neurodegenerative diseases of the CNS

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Abstract

The clinical manifestation of most diseases of the central nervous system results from neuronal dysfunction or loss. Diseases such as stroke, epilepsy and neurodegeneration (e.g. Alzheimer's disease and Parkinson's disease) share common cellular and molecular mechanisms (e.g. oxidative stress, endoplasmic reticulum stress, mitochondrial dysfunction) that contribute to the loss of neuronal function. Neurotrophic factors (NTFs) are secreted proteins that regulate multiple aspects of neuronal development including neuronal maintenance, survival, axonal growth and synaptic plasticity. These properties of NTFs make them likely candidates for preventing neurodegeneration and promoting neuroregeneration. One approach to delivering NTFs to diseased neurons is through viral vector-mediated gene delivery. Viral vectors are now routinely used as tools for studying gene function as well as developing gene-based therapies for a variety of diseases. Currently, many clinical trials using viral vectors in the nervous system are underway or completed, and seven of these trials involve NTFs for neurodegeneration. In this review, we discuss viral vector-mediated gene transfer of NTFs to treat neurodegenerative diseases of the central nervous system.

1. Introduction

A virus can be viewed as proteinacious, lipidic capsule containing RNA or DNA and capable of delivering that nucleic acid to the nucleus of an infected cell. In this view, the evolutionary accomplishments of a virus to deliver selected genetic material to cells of interest can be exploited for drug delivery. The concept of converting a virus into a gene delivery vehicle or vector has been extensively investigated for nearly three decades. Viral vectors are now routinely used as tools for studying gene function as well as developing gene-based therapies for a variety of diseases. There are eighteen clinical trials using adeno-associated viral vectors and one trial using lentiviral vector for diseases of the central nervous system (CNS) currently underway or completed (Table 1). In this article, we will review the preclinical and clinical data from studies of viral vectors used to deliver genes encoding neurotrophic factors (NTFs) to the central nervous system for the treatment of neurodegenerative diseases.

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2. Neurotrophic Factors (NTFs)

Neurotrophic factors are typically small (<20 kD) secreted proteins that regulate multiple aspects of neuronal development including neuronal maintenance, survival, axonal growth and synaptic plasticity. Neurotrophic factors can be administered exogenously either as recombinant proteins or via gene delivery. Based on animal models of neurological disease, administered NTFs can prevent or slow the disease process by enhancing metabolism, growth and function of neurons. The classical NTF hypothesis is that the survival of developing neurons depends on the limited supply of the neurotrophic factor produced by target fields (1). Originally, the NTF hypothesis was proposed based on studies of neurons from the peripheral nervous system, but further research has proven that the hypothesis holds true for the central nervous system (CNS) as well (2). The original hypothesis has been expanded to show that trophic factors are important for neuronal phenotype and are delivered from not only the target, but from a sundry of sources (Figure 1). The delivery of NTFs has been postulated as a therapy for diseases in which neuronal function is disturbed, such as spinal cord injury, epilepsy, neurodegenerative disorders (Parkinson's disease, Alzheimer's disease, amyotrophic lateral sclerosis, Huntington's disease), neuropathic pain and stroke. Neurotrophic factors can also prevent nerve cell death caused by various insults, including nerve injury and brain trauma. In this review, we will focus on proteins that have been recognized as neurotrophic factors *in vivo* and have been delivered by viral vectors for testing in animal models or clinical trials for neurodegenerative diseases. Two families of neurotrophic factors that we will discuss are the neurotrophins, a family of structurally-related secreted proteins, consisting of nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) and NT-4/5, and the transforming growth factor β (TGF- β) superfamily which includes glial cell line-derived factor (GDNF) and its homolog, neurturin (NTN). Effects of neurotrophic factors vary between families and between members of the same family, but they share the overall mechanism of secretion, receptor binding and intracellular signaling leading to altered cellular processes (Figure 2). In this review, we will focus on the use of neurotrophic factors in gene therapy of neurodegenerative diseases.

3. Viral Vectors

One approach to supply neurotrophic factors to diseased neurons is through viral vector-mediated gene delivery. Viral vectors can be directly placed in the region of interest to transduce cells that will secrete the NTF to act on NTF-receptor containing neurons (Figure 3). There are many viral vectors capable of gene delivery in the CNS and they vary in their structural and biochemical properties. For *in vivo* gene therapy in neurodegenerative diseases, we review application of four viral vector systems: Adenovirus, adeno-associated viruses, herpes simplex virus, and lentivirus.

In addition to delivering DNA directly to the cells of the CNS for *in vivo* gene therapy, the *ex vivo* approach is also viable and clinically relevant (3,4). *Ex vivo* gene therapy uses cells, often host-derived, that have been genetically modified *in vitro* using a viral vector such a lentivirus or transfection of transgenic DNA to secrete therapeutic gene products or neurochemicals (3). For this review, we focus on the *in vivo* gene therapy approaches in the CNS.

3.1. Adenovirus (Ad)

The adenovirus (Ad) vector is promising as a gene delivery vehicle for human gene therapy as illustrated by its predominant use in clinical trials for human disease (Figure 4). The Ad vectors are non-enveloped virions with a double-stranded DNA genome of 26–45 kb. They have capacity for 4.5 kb to 30 kb of foreign DNA, which accommodates most of the known cDNA sequences and facilitates insertion of multiple expression cassettes. The Ad can be purified and concentrated up to 10^{12} – 10^{13} viral particles/ml. This feature makes it a useful vector system

for gene therapy applications requiring high viral doses. There are over 51 Ad serotypes in humans (5), most using the widely-distributed coxsackie and adenovirus receptor (CAR) for primary attachment to the host cell (6,7), and αv integrin for cell entry (8). Adenovirus vectors have a broad tissue tropism, or host cell range, including post-mitotic cells such as neurons. Recent research suggests that major histocompatibility complex I (MHCI) and sialic acid residues also function as adenovirus receptors (8,9). Recently, Ad serotype 35 (Ad35) vectors have been developed (10). These vectors exhibit a broad tropism for human cells due to ubiquitous expression of their primary receptor, CD46 (11–13) but their use in the CNS has not been exploited.

Two types of Ad vectors have been extensively examined for gene transfer in CNS, recombinant and “gutless” vectors. The Ad genome has five early genes: E1A, E1B, E2, E3, and E4. These early genes are involved in the activation of viral DNA replication and the expression of viral structural proteins. In recombinant Ad vectors, portions of the early adenoviral genes are replaced with the transgenic DNA of interest. The first generation of Ad vectors was devoid of only one or two viral early genes (E1 and/or E3), which impaired their ability to replicate. Although the E1 gene was deleted, the first generation Ad vectors replicated and expressed other adenoviral genes at decreased levels compared to a wild-type virus, inducing strong cellular immune response in the brain (14,15). This hampered the use of the first generation of Ad vectors for long-term neurological gene therapy applications. The second and third generations of Ad vectors contain additional deletions in genes E2 and/or E4 which increase transgene capacity and decrease immunogenicity compared to first generation vectors (16–18). Gutless adenoviral vectors only contain a packaging signal and can accommodate up to 37 kb of foreign DNA (19,20). These vectors have improved the stability of transgene expression by eliminating the expression of viral proteins and diminishing the potential immune response (21,22). However, these vectors still induce a capsid-mediated inflammatory response in the rat brain (23,24), suggesting the use of these vectors requires additional caution.

Overall, the development of recombinant and gutless Ad vectors has improved its efficiency and usefulness in treating CNS. Although Ad vectors have been used the most of all viral vectors for gene therapy clinical trials, currently, there are no clinical trials using adenoviral vectors in the CNS for neurodegeneration. Further studies on the efficacy and safety of newer generations of Ad vectors are needed to determine their potential for human gene therapy of CNS diseases.

3.2. Adeno-associated Virus (AAV)

The emergence of AAV at the forefront of gene delivery for diseases of the CNS is exemplified by its use in 18 clinical trials (Table 1) and can be attributed to its biological properties (Table 2). The wild-type AAV genome is a single-stranded DNA molecule (~4.7 kb) comprised of two open reading frames encoding the four replication and packaging function genes (*rep*) and three capsid (*cap*) genes flanked by inverted terminal repeats (ITRs). The *rep* and *cap* genes alone are insufficient to cause a productive infection; instead, a helper virus, such as adenovirus or herpes virus, is required to produce infective AAV particles. An infective particle then enters a host cell by receptor-mediated endocytosis and trafficking to the nucleus where the viral genome integrates into the host genome at a specific location on human chromosome 19q13.4. To date, no disease or pathology has been attributed to the presence of AAV, and this feature of AAV makes it an attractive vector for gene therapy.

Adeno-associated virus was first used as a viral vector for CNS gene delivery by replacing the *rep* and *cap* genes with *E. coli LacZ* gene and using adenovirus as a helper virus (25). The first generation of AAV vectors was helper-virus-based, but several groups developed a “helper-free” system where the helper genes could be provided by transfection or stable cell lines (26–30). Using a helper-free system, only the two ITR DNA elements are required for

packaging. This allows for up to 6 kb of vector genome DNA for incorporating a promoter, cDNA and poly-adenylation signal (31). One of the more recent advances in AAV vector biology has been the development of double-stranded DNA or self-complementary AAV (scAAV) vectors by deleting the terminal resolution sequence (*trs*) in one ITR (32,33). The *trs* deletion stabilizes AAV as a double-stranded replication intermediate, which negates the need for second strand replication, a major limitation of transduction efficiency. While the scAAV vector exhibits a more rapid onset of transgene expression, the transgene capacity for a scAAV vector is only half of a single-stranded DNA vector. However, the scAAV vectors do offer high efficiency of transduction and rapid transgene expression for relatively small genes, as do most neurotrophic factors.

The tropism, or host cell specificity of AAV is determined by the three *cap* genes. Natural or synthetic variations in the *cap* genes have led to the identification of over 100 serotypes or hybrid serotypes (34,35). The tropism of an AAV vector can be varied simply by exchanging the *rep/cap* gene source during viral transduction. Serotype 2 is the predominantly studied serotype in AAV vector biology and gene therapy development, however, many cells or tissues are not efficiently transduced by serotype 2 vectors. Nevertheless, many other serotypes of AAV have been shown to transduce cells of the CNS such as neurons and glia (36–43), illustrating a capacity to deliver neurotrophic genes to regions susceptible to neurodegeneration. The availability of over 100 serotype variations expands the tropism of AAV beyond serotype 2. In addition, having antigenically distinct serotypes may allow for repeated vector administration in humans by avoiding or reducing the immune response to previously injected vector or to an established AAV infection.

Once an AAV vector has transduced a cell, the viral genome enters the nucleus where it exists primarily as double-stranded circular episomes or as high molecular weight concatamerized episomes. However, recent evidence has shown that random integration of AAV vectors is associated with tumorigenesis in mice (44). The integration frequency of AAV vectors into host cell genomes may be cell-type dependent and requires further investigation.

Overall, AAV vectors are capable of delivering transgenes to the CNS for evaluating the therapeutic potential of neurotrophic factors in several models of neurodegeneration. AAV is currently the predominant vector for human gene transfer trials that focus on neurodegeneration (see Table 1).

3.3. Herpes Simplex Virus (HSV)

Herpes Simplex Virus-1 has a double-stranded DNA genome enclosed in a protein capsid surrounded by a lipid membrane envelope. The capsid and envelope are separated by a tegument containing viral and cellular proteins. The 152 kb genome of HSV-1 is the largest and most complex of all the viruses being developed for gene therapy. HSV-1 is neurotrophic and can establish lifelong presence in sensory neurons. Due to this natural tropism, the majority of gene transfer applications of HSV-1 vector have been directed toward the nervous system (45–48).

Two types of HSV-1 vector systems have been developed, recombinant virus and amplicons. Recombinant viral vectors are generated by replacing nonessential viral genes with a transgene (s) of interest at different sites in the viral genome; these vectors can usually accommodate up to 40 kb of transgenic DNA. Recombinant virus can be divided into two systems, replication-competent attenuated vector and replication-incompetent attenuated vector. Replication-competent attenuated vectors still contain essential genes for *in vivo* replication whereas replication-incompetent vectors are created by deleting genes that are required for replication. Replication-competent viruses have limited therapeutic utility, such as in cancer therapies, where viral replication and toxicity is restricted to the dividing cancer cells of a tumor. These

recombinant viral vectors also can be used as helper virus for the production of amplicon vectors. However, recombinant vectors exhibit cytopathic effects due to the presence of immediate early (IE) gene products (49). Recombinant vectors, lacking multiple IE genes, exhibit diminished toxicity, but result in severely reduced transgene expression (50,51).

The HSV-1 amplicon is similar to the adenoviral gutless DNA vector system where only a replication and packaging DNA sequence is present in the viral-vector genome. Advantages of using amplicon are reduced cellular toxicity and low immune response compared to the recombinant HSV-1 vectors (52) and its capacity to carry large fragments of foreign DNA. This latter feature allows for not only the insertion of entire genomic loci but also the addition of various elements, such as promoters, inducible systems for regulated gene expression or several separate expression cassettes (reviewed in (53)).

Use of HSV vectors in CNS clinical trials has been limited to brain tumor therapy (reviewed in (54)), and HSV-1 amplicon vectors have not been tested in human clinical trials. However, the potential of HSV vectors for CNS gene therapy have been demonstrated in animal studies (55–59), and they continue to be useful tools for gene delivery to the CNS. Overall, the HSV vector system offers neuronal transduction with a large transgene capacity that allows for cell specific and/or regulated gene expression of multiple genes.

3.4. Lentivirus (LV)

Lentiviruses are a subfamily of retroviruses capable of integrating their genome into both mitotic cells, such as glia, and post-mitotic cells, such as neurons, which makes them of clinical interest for treating neurodegenerative diseases. One clinical trial using a lentiviral vector to treat Parkinson's disease (PD) is currently under way (Table 1). The LV genome is a single-stranded RNA in the sense orientation (~9.7 kb) that encodes cis-acting sequences for packaging, reverse transcription, nuclear localization and integration, as well as 9 genes encoding structural (gag, pol and env), regulatory (rev and tat) and accessory (vpu, vpr, vif, and nef) proteins.

The first generation of LV vectors based on the human immunodeficiency virus 1 (HIV-1) was generated using a three-plasmid system consisting of a packaging plasmid, vector plasmid and envelope plasmid (60). The packaging plasmid contains the viral genes under the CMV promoter except for the env and vpu open reading frames (ORFs), which have been deleted and provided by the envelope plasmid that encodes vesicular stomatitis virus G protein (VSV-G) in place of the HIV-1 env proteins. The vector plasmid contains HIV-1 cis-acting elements that enable the transgene and its promoter to be packaged. Naldini et al.(60) demonstrated that the gene encoding *E. coli* beta-galactosidase or *P. pyralis* luciferase can be successfully packaged and used to stably infect neurons in the rat brain. Second generation (61) and third generation (62,63) packaging systems for LV have been developed that remove additional viral sequences from the vector plasmid to further decrease the probability of producing replication-competent recombinant particles. Lentiviral vectors can accommodate a wide range of transgenic DNA (up to 18 kb proviral length), however substantial titers are achieved when the proviral DNA is between 6–9 kb (64). Further modifications have also been made to increase packaging efficiency (reviewed in (65,66)).

The tropism of “wild-type” HIV-1 envelope proteins is predominantly for cells of mononuclear phagocyte lineage (67,68). Pseudotyping LV vectors with VSV-G provides a broad tropism that includes neurons and glia (60,69). The VSV-G protein stabilizes the viral particle enabling concentration by centrifugation (70), which is necessary for *in vivo* CNS applications. Pseudotyping with the envelope proteins of murine leukemia virus (MuLV) or the rabies-related Mokola virus are also capable of transducing cells in the mouse CNS (71). In addition to HIV-1-based vectors, other LV vectors based on non-primate (equine, feline and bovine

immunodeficiency viruses or EIAV, FIV and BIV, respectively) and primate (simian immunodeficiency viruses or SIV) lentiviruses have been developed and are amenable to pseudotyping for neuro- and gliotropism (72,73).

The application of lentiviral vectors for *ex vivo* gene delivery of neurotrophic factors also holds promise (74). The integration properties of lentiviral genome into the host genomes allow for stable transduction of cell lines, primary cells or stem cells. Such cells, designed to restore neural function and prevent neurodegeneration can be transplanted or further differentiated *in vitro* prior to transplantation. Clinical trials using *ex vivo* therapy with lentiviral vectors are currently underway for non-CNS diseases (<http://www.gemcris.od.nih.gov/>).

The random integration of a lentiviral vector genome into the host cell genome leading to an oncogenic mutation is the primary safety concern for its use in gene therapy. Recent studies have identified non-integrating lentiviral (NIL) vectors that are deficient in integrase activity, the enzymatic activity required to catalyze integration into the host cell genome (75). The NIL genome exists as a linear or circular double-stranded DNA molecule in the nucleus of transduced cells (76). Using a NIL vector, successful transduction of the rat striatum and retrograde transport to the dopaminergic neurons of the substantia nigra have been demonstrated (77). Transduction of additional brain regions by *in utero* delivery of NIL to mice was also shown. Future studies evaluating NIL for gene delivery of NTFs in models of degeneration will determine the therapeutic potential of this vector.

Overall, lentiviral vectors offer long term, neuronal transgene expression with minimal inflammatory response (72). Although insertional mutagenesis remains a primary safety concern, an on-going clinical trial using lentiviral delivery for Parkinson's disease shows promising results with no reported evidence of tumorigenesis (Oxford Biomedical Press Release, 7/13/2009). Once NIL vectors are evaluated in models of degeneration, their use in clinical setting may become more prominent if higher safety profile is conferred.

4. Neurodegenerative disorders and gene therapy

4.1. Alzheimer's disease (AD)

Alzheimer's disease is a multifactor, neurodegenerative disorder associated with progressive functional decline, dementia and neuronal loss that is initiated in specific regions and progresses in a specific manner. In the United States, there are about 2.4 to 4.5 million people living with AD (78). The most critical element for decline in memory and cognition in AD appears to be loss of the basal forebrain cholinergic neurons (BFCNs) of the nucleus basalis of Meynert (NBM) (79–81). Nerve growth factor (NGF) is the first neurotrophic factor identified for these neurons (82). In AD, loss of mature NGF is observed in the basal forebrain (83,84). In the absence of NGF, cholinergic neurons exhibit shrinkage, reduction in fiber density and down-regulation of transmitter-associated enzymes, resulting in a decrease of cholinergic transmission (85). The administration of NGF into the adult rat brain prevents the death of basal forebrain cholinergic neurons both spontaneously and after injury (86–89). NGF administration also improves learning and memory in lesioned and aged rats (86,90–94), and reverses basal forebrain cholinergic neuronal atrophy and improves neuronal function in an amyloid overexpression model of AD (95,96).

NGF does not cross the blood-brain barrier when administered systemically (97) and has a short half-life; therefore, it must be administered directly into the brain. In an initial trial of NGF in AD patients, continuous infusion of NGF into the cerebral ventricles was tested, but due to the adverse side-effects that occurred in subjects, including pain and weight loss, the trial was terminated without obvious benefits (98,99).

Towards clinical use of NGF for AD, *ex vivo* preclinical studies demonstrated controlled and sustained release of NGF with no associated toxicity (93,100–103). In 2001, a Phase I trial of *ex vivo* NGF gene delivery was initiated in 8 subjects diagnosed with early-stage AD (4). Fibroblasts obtained from skin biopsies in each subject were genetically modified using retrovirus to express human NGF and implanted into the basal forebrain. After five years, no adverse events related to either NGF or the presence of the viral vector was noted. Serial positron emission tomography scans in four subjects showed a significant increase in metabolic activity throughout the cortex, consistent with widespread modulation of cortical activity by NGF in the Nucleus basalis. Cognitive testing suggested slowing of cognitive decline, but reliable conclusions could not be drawn from the small sample size. The *ex vivo* NGF trial has shown positive effects on brain metabolism by PET scans for 18-glucoseoxyglucose, and on cognition by Mini-Mental Status Examination and Alzheimer's Disease Assessment Scale-Cognitive subcomponent analyses with no long-term adverse effects. These data support the therapeutic approach of using elevated NGF locally in the brain for treating AD. *In vivo* gene delivery of NGF by AAV (104,105), adenovirus (106) or lentiviral vector (107,108) provided sustained expression with beneficial effects in animal studies. A Phase I clinical trial of AAV-NGF gene delivery in early to mild stage AD patients demonstrated that AAV-NGF was safe and well tolerated and cognitive testing suggested a reduced rate of cognitive decline (109). A Phase II multicenter, sham surgery-controlled trial of AAV-NGF (CERE-110) gene transfer is in progress (Table 1, NCT00876863 or US-930;110). The *in vivo* delivery of NGF may prove to be an effective way to deliver sustained NGF to the brains of AD patients and attenuate neurodegeneration.

4.2. Amyotrophic lateral sclerosis (ALS)

Amyotrophic lateral sclerosis is a neurodegenerative disease that results from the progressive loss of motor neurons in brain and spinal cord. Clinical studies using intrathecal BDNF protein to treat ALS failed to show improvement in Phase III clinical trials (111). These studies were initiated in the early 1990's when viral vector-mediated gene transfer was still in early development. Subsequent preclinical studies suggest other neurotrophic factors may be beneficial in treating ALS. Using a rodent model of ALS, *ex vivo* delivery of GDNF via myoblasts transduced with a retroviral vector and transplanted into hind limb muscles increased motor neuron survival and delayed the "ALS-related" decline in performance on motor tests (112). Adeno-associated viral vector-mediated delivery of GDNF to the neuromuscular junction also shows evidence of retrograde transport of GDNF to motor neurons and impedes neurodegeneration in a transgenic model of ALS (113). Intramuscular delivery of an adenovirus expressing NT-3 improved life span and neuromuscular function in a mouse mutant progressive motor neuropathy model (114). The most promising factor for treating ALS is not a neurotrophin or a TGF-beta family member, but insulin-like growth factor 1 or IGF-1 (115). Preclinical studies using AAV-IGF1 in rodent models of ALS have shown a significant reduction in motor neuron loss and improvement in behavior (116–119). Early clinical trials using subcutaneous IGF-1 protein show mixed results in efficacy (120,121). As the safety and efficacy of AAV as a vector is established, future studies examining vector-mediated delivery of IGF-1 may prove to be more efficacious than recombinant protein delivery for treating ALS. This is really unclear. Lastly, additional proteins with neurotrophic activity in motor neurons may be useful for treating ALS, but require further investigation in animal models of ALS (122).

4.3. Huntington's disease (HD)

Huntington's disease is a progressive neurodegenerative disease with no effective treatment currently available. Gene delivery studies using AAV, adenovirus and lentivirus have demonstrated neuroprotection in several animal studies (123–127). Adeno-associated virus expressing GDNF or neurturin was found to be neuroprotective in a 3-nitropropionic acid rat

model of HD (123,126). In a quinolinic acid rat model of HD, GDNF and BDNF were neuroprotective when delivered into the striatum 3 weeks before the toxin (128). In addition, AAV expressing neurturin or GDNF in a transgenic mouse model of HD reduced neurodegeneration, and GDNF delivery by AAV ameliorated behavioral deficits (125,129). An adenovirus used to deliver BDNF was found to be neuroprotective in a study where virus was injected 2 weeks before quinolinic acid lesioning (130).

In a rat study where tetracycline-regulated lentiviral vector-mediated delivery of ciliary neurotrophic factor (CNTF) was used, CNTF expression protected striatal neurons from quinolinic acid toxicity and decreased behavioral deficits (127). Also, adenovirus-mediated gene delivery of CNTF has been shown to be neuroprotective in the 3-nitropropionic acid HD model (124). A Phase I clinical trial demonstrated that intracerebral delivery of CNTF by an encapsulated cell line genetically modified to secrete CNTF showed no signs of toxicity (131). These results suggest that over-production of CNTF may be safe and tolerable, but due to variability in the CNTF levels, further studies are needed to establish dosing of CNTF for the effective treatment of HD. Indeed, the treatment of HD using viral vectors expressing neurotrophic factors such as CNTF, GDNF or NTN may be feasible based on similar clinical studies of an AAV vector expressing NTN (CERE-120) for Parkinson's disease demonstrating that the overall approach is safe and well tolerated (132,133). The same AAV-NTN vector, CERE-120, has recently been shown to reduce striatal neuron loss in a rodent model of HD (131).

4.3. Epilepsy

Epilepsy is a neurological disorder characterized by recurrent episodes of seizures. The roles of neurotrophic factors in seizure activity have indicated both pro-and anti-epileptic effects (134). For example, over-expression of BDNF by AAV increased seizure activity in rat (135), whereas overexpression of GDNF by AAV (137) or Ad (138) decreased seizure frequency and severity in rodent models of epilepsy. Continuous intraventricular infusion of NT3 can also reduce seizures in the rodent model (136). The differential effects of neurotrophins and the GDNF family members in models of epilepsy have been described (136,139) and underscore the need for further investigation into their therapeutic potential for treating epilepsy. Currently, there is a proposed clinical trial using AAV to delivery neuropeptide Y for the treatment of epilepsy (www.gemcris.od.nih.gov; OBA#669).

4.4. Parkinson's Disease (PD)

Parkinson's disease is a slowly progressive disorder with no known single etiology. The predominant pathological feature is the loss of the dopaminergic neurons in the substantia nigra that project to the striatum (nigrostriatal pathway). The well established link between selective degeneration of the nigrostriatal dopaminergic neurons and the neurological deficits in Parkinson's patients provides defined targets for neurotrophic-factor-based gene therapy. In fact, PD is the most extensively studied neurodegenerative disease and model for gene-based therapies using neurotrophic factors (>50 preclinical studies using animal models and 9 clinical trials). Following the outline of approaches for viral vector delivery (Figure 3), a viral vector and its encoded transgene can be targeted to the dopaminergic cell bodies in the substantia nigra or the dopaminergic nerve terminals in the striatum; both approaches continue to be investigated preclinically and clinically.

Although striatal delivery of BDNF by AAV (57) or HSV (59) offers modest improvement in a rat model of PD, members of the GDNF family have proven most beneficial and have emerged in clinical trials. Neuroprotective and neurorestorative effects for GDNF have been demonstrated using AAV in rodents (140–145), AAV in non-human primates (146–150), LV in rodents (151–156), LV in non-human primates (145,157), Ad in rodents (158–167) and HSV

in rodents (58,59). Of note, two studies using a LV vector in a rodent model of PD showed that long term overexpression of GDNF may decrease TH expression and activity (168,169). Despite the overwhelming animal model data supporting the use of GDNF gene-based therapy for PD, there are no active clinical trials. Part of this disconnect stems from halted clinical trials that showed mixed results from infusions of GDNF protein in PD patients (170). Technical, ethical and legal issues must be resolved before the GDNF-based therapies can be developed; however, much progress has been made with NTN, the homolog of GDNF.

Neurturin shares 42% protein homology with GDNF, uses similar signaling pathways (171), and promotes survival of dopaminergic neurons (172). AAV-mediated delivery of NTN in a rodent model of PD, demonstrated that bioactive NTN is stably expressed, neuroprotective and shows no adverse effects (173,174). Primate studies using AAV-NTN also show long-term expression, neuroprotection, enhanced dopaminergic activity and no adverse side effects with a wide safety margin of dosages (175–178). Safety, dosing and tolerability of intraputamenal delivery of AAV-NTN (CERE-120) were demonstrated in humans (132,133). However, Phase II testing did not show an increase in the primary endpoint of improved Unified Parkinson's Disease Rating Scale (UPDRS) motor off score at 12 months though CERE-120 conferred a significant improvement in the UPDRS ($p=0.025$) and in secondary motor measures ($p<0.05$) at 18 months (Ceregene, Press Release 11/26/2008). The study conducted by Ceregene, Inc. is ongoing and will collect additional time points for evaluating efficacy of treatment. The AAV-NTN clinical studies along with studies of an AAV vector expressing glutamate decarboxylase 65/67 (GAD65/67) (179,180) and the aromatic L-amino acid decarboxylase (AADC) (181) studies are establishing gene therapy protocols for Parkinson's disease.

4.5. Stroke

Stroke is the third leading cause of death and disability of humans. Current strategies available for the treatment of ischemic injury are largely based on intravascular thrombolysis and on the ability to identify patients early. The thrombolytic approach is not uniformly successful and, in order to be a successful treatment, needs to be within the short optimal time window. Post-stroke physiotherapy plays an important part in patients' recovery from an ischemic brain injury, which suggests that functional recovery of neuronal networks occurs. Although current preclinical studies show neuroprotective effects of neurotrophic factors present at the time of stroke, delivering neurotrophic factors by viral-vector mediated gene delivery may augment recovery processes that would rely on synaptic plasticity, neuronal differentiation and axonal outgrowth.

Several viral vectors have been used to deliver genes encoding neurotrophic factors to animals with ischemic brain injury. Intracortical administration of an AAV vector expressing GDNF during a 90 min middle cerebral artery occlusion (MCAo) decreased infarction volume and TUNEL staining (182). An Ad vector containing GDNF gene injected into the lumbar spinal cord two days before spinal cord ischemia in a rabbit decreased TUNEL staining and caspase-3 immunoreactivity in motor neurons (183). Also, 7 days after an ischemic insult, GDNF-treated animals had increased motor neurons and improved neurological outcome compared to controls (183). Intraventricular delivery of Ad-GDNF two days before a 5 min bilateral common carotid arterial occlusion prevented the loss of hippocampal neurons (184).

Using an ischemic injury model in rodent brain, intracortical delivery of an HSV-amplicon-based vector encoding GDNF four days before MCAo was found to decrease cortical infarction volume, promote behavioral recovery and decrease caspase-3 immunoreactivity (56). However, an HSV amplicon encoding GDNF failed to promote recovery when delivered intracortically into the ischemic site three days after injury (56).

Although there is accumulating evidence that neurotrophic factors such as GDNF (185) may be therapeutically useful if present prior or acutely following an ischemic event, the ability to use neurotrophic factors for treating stroke will likely be predicated on the development of non-neurosurgical approaches to administer viral vectors acutely following a stroke. The transient breakdown of the blood brain barrier in the ischemic region may provide an opportunity to deliver therapeutic genes using vectors capable of rapid transgene expression. Further studies on methods of vector delivery in models of stroke will be useful in determining the utility of gene-based therapy using neurotrophic factors for human ischemic diseases.

5. Moving from “Bench to Bedside”

To treat neurodegenerative disorders, the move of viral vector-mediated delivery of neurotrophic factors from bench to bedside depends upon knowledge gained from preclinical studies; evaluation of patient safety based on early clinical trials; identifying enhanced procedures for early diagnosis of degenerative diseases; developing non-invasive delivery methods; defining and understanding the activity of NTFs in the target treatment area; and finally in addressing ethical issues related to gene therapy for neurodegenerative disorders.

Despite the wealth of preclinical studies demonstrating therapeutic potential of viral vector-mediated delivery of neurotrophic factors in models of neurodegeneration, only AAV-NGF and AAV-NTN are being evaluated for Alzheimer’s disease and Parkinson’s disease, respectively (see Table 1). The overall number of clinical trials worldwide using AAV, Ad, HSV or LV viral vectors for human disease has gone from the first 5 proposed trials in 1994 to a cumulative 465 proposed, open or completed trials in 2009 (Figure 4). As Phase I trials are completed, data on safety, dosing and tolerance of the viral vectors in the CNS are being used to develop safer, more efficient vectors. As knowledge of vector biology is gained, clinical investigations will need to modify endpoint measures used to evaluate safety in humans. For example, a recent study found that a therapeutic transgenic cassette in AAV currently being used in a clinical trial has alternative reading frames (ARF) that create antigens capable of inducing a cytotoxic T lymphocyte response to the cells transduced with the “therapeutic gene” (186). In this example, preclinical testing of viral vectors for production of ARFs is vital prior to usage in clinical trials. For gene therapy to ultimately be a standard practice, patient safety must remain the foremost concern.

An essential step to the effective implementation of routine NTF gene therapy is early diagnosis of neuronal dysfunction and loss. Neuroimaging and systemic monitoring of biomarkers for genetically predisposed or symptomatic individuals will be paramount to the success of the therapy. Towards this goal, over 100 clinical trials are ongoing or completed for Parkinson’s disease and Alzheimer’s disease alone (www.clinicaltrials.gov). Establishing the stage of a neurodegenerative disorder will aid in determining whether a neurotrophic factor needs to be neuroprotective and slow or prevent neurodegeneration or neuroregenerative and promote or augment endogenous mechanisms of restoring neuronal connectivity and function. The first goal of a gene therapy should be to slow down or prevent the progression of the disease. The second goal is to promote regeneration of lost neurons and neuronal connections. Such decisions will rely on preclinical data of the molecular and cellular mechanisms of NTFs and their receptors.

Once a disease is diagnosed and a gene therapy chosen, the vector must be delivered to the appropriate brain region. Preclinical studies often use invasive procedures to demonstrate efficacy, but such procedures can be impractical to implement in humans. Current human trials on viral gene delivery to the brain still rely on invasive surgical procedures, so developing minimally invasive procedures for gene delivery while still ensuring sufficient transduction of the target area or site of NTF release is of great interest. For example, image-guided,

convection-enhanced delivery (CED) of viral particles to increase the efficacy of a single viral injection is a promising less-invasive procedure (187). In this same vein, vectors capable of delivering genes to the CNS via injection into the cerebrospinal fluid or blood would prove most valuable. Research on vector engineering, both viral and non-viral, will lead to more specific transgene targeting and expression. Ideally, a vector should be capable of crossing the blood brain barrier (such as AAV serotype 9 (188)), then using a cellular receptor for cell-specific entry and finally delivering transgenic DNA that contains a drug-regulated, cell-restricted promoter to express a therapeutic gene.

While trying to minimize invasiveness of the procedure and maximize delivery of NTF to the diseased target area, the activity of neurotrophic factor must be considered. In order for viral vector delivery of NTFs to be therapeutic for neurodegeneration, cells need to be healthy enough to 1) support NTF production and release at the target site, and 2) support the expression and signaling of the cognate NTF-receptor at the target site. Studies devoted to identifying radioligands capable of monitoring NTF receptor availability in the diseased tissue region are needed for evaluating the outcome of clinical trials. Clinical trials must be designed to address any failures to meet primary endpoints. A sound clinical trial must be able to distinguish between a failure of NTF to alter the disease progression versus a failure of technique (such as insufficient dosing, insufficient coverage of target area, and diminished transgene expression or receptor expression over time). Studies must address issues such as receptor down-regulation, i.e. does the NTF receptor get down regulated when its ligand NTF is over expressed by a viral vector? Does overexpression of a NTF down-regulate essential cellular genes (189) or vector-mediated gene expression that may negatively impact the disease outcome? Studies must also reflect sufficient sample sizes to support any positive or negative findings.

Finally, irrespective of the scientific issues, there are ethical issues regarding clinical trials that must also be addressed. Is an individual with cognitive impairment due to a neurodegenerative disease able to make informed consent for a clinical trial? Is it fair that they be excluded from these trials? Should placebo trials be conducted when an invasive surgery is involved?

6. Conclusion

In this review, we describe preclinical studies that demonstrate the versatility and efficiency of viral vectors for delivering neurotrophic factors to the CNS. The existence of clinical trials for viral-vector-mediated gene delivery in the CNS illustrates the progress being made towards gene therapy for neurodegenerative diseases of the CNS. The current preclinical and clinical studies using gene therapy for diseases of the CNS are establishing the foundational protocols on which to develop more sophisticated therapies that combine multiple genes with pharmacological manipulation to create a “pharmacogene” therapy for treating human diseases.

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A. Classic Neurotrophic Model

B. Current Neurotrophic Model

Figure 1. Models of neurotrophic factors secretion and site of action

Neurotrophic factors were initially identified for their ability to promote neurite outgrowth. NGF was the first neurotrophic factor identified (82,190) and subsequent studies led to the “neurotrophic hypothesis” which states that developing neurons compete with each other for a limited supply of NTF provided by the target cell. (A) The innervating cell stimulates release of a NTF from the target cell and reinforces the interaction of the innervating neurite and the target cell. (B) Additional proteins with neurotrophic activity have subsequently identified to be secreted in both target-derived and non-target derived fashion to have autocrine or paracrine effects. Both neurons and glia can produce NTFs and the expression of specific NTF receptors determines a cell’s response to the NTFs present in the extracellular environment.

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Figure 2. Neurotrophic factors act on receptor kinases to exert effects

Neurotrophic factors released into the extracellular environment bind to their cognate receptor or family of receptors with differing affinity. A) The NTF/receptor interaction can activate intracellular signaling via A) phosphorylation by a transmembrane coreceptor (e.g. GDNF) or B) phosphorylation by receptor dimerization (e.g. NGF). Receptor phosphorylation leads to the activation of various signaling pathways that have multiple effects on the cell including transcriptional activation, altered cellular metabolism and synaptic plasticity. For more details on TGF-beta receptors and neurotrophin receptor signaling see reviews (191–194).

Figure 3. Directing neurotrophic factors to the region of diseased neurons

The tissue location of NTF production by a viral vector is determined by NTF receptor expression on diseased neurons (cell body versus processes) and tropism of the viral vector being used. Vectors can be delivered to the cell body (A-left) or neuritic processes (A-right) of the diseased neurons. (B) Viral vector targeting to the region of the neuron body can directly transduce the diseased neuron, glia or other neurons for secretion of the therapeutic NTF. (C) Viral vector placed in the region of the neuritic processes of the target neuron can lead to retrograde delivery of the vector genome to the nucleus of the target neuron (dashed line) or transduce glia or cells innervated by the diseased neuron to secrete the therapeutic NTF.

Figure 4. Worldwide clinical trials involving Ad, AAV, HSV or LV

(A) Total clinical trials for Ad, AAV, HSV and LV by year. (B) Total clinical trials for each vector by year. Trial numbers represent proposed, open, completed or discontinued trials listed at <http://www.wiley.co.uk/genmed/clinical/> as of October 2009.

Table 1
Clinical trials of gene therapy using viral vectors for neurodegenerative diseases in the CNS

Condition	Identifier ^{A,B}	Viral Vector	Gene(s)	Status ^C	Phase	Primary Sponsor	Location
Alzheimer's disease	NCT00017940 ^A , US-322 ^B	retrovirus (<i>ex vivo</i>)	Nerve Growth Factor (NGF) / CERE-110	Completed (4)	I	The Shiley Family Trust	USA
	NCT00087789 ^A US-623 ^B	AAV Serotype 2	Nerve Growth Factor (NGF) / CERE-110	In Progress, not recruiting - March 2009 ^D	I	Ceregene	USA
	NCT00876863 ^A US-930 ^B	AAV Serotype 2	Nerve Growth Factor (NGF) / CERE-110	Actively recruiting - September 2009 ^D	II	Ceregene	USA
	US-669 ^B	AAV 1/2	Neuropeptide Y	N/A	I	N/A	USA
Parkinson's Disease	NCT00252850 ^A US-689 ^B	AAV Serotype 2	Neurturin (NTN) / CERE-120	Completed March 2007 (133)	I	Ceregene	USA
	NCT00400634 ^A US-788 ^B	AAV Serotype 2	Neurturin (NTN) / CERE-120	Completed November 2008 ^D	II	Ceregene	USA
	NCT00985517 ^A US-981 ^B	AAV Serotype 2	Neurturin (NTN) / CERE-120	In Progress, Actively recruiting - September 2009 ^D	I/II	Ceregene	USA
	ES-008 ^B	AAV Serotype 2	Neurturin (NTN) / CERE-120	Open, Date approved/initiated September 2008	II	N/A	Spain
	SE-006 ^B	AAV Serotype 2	Neurturin (NTN) / CERE-120	Open, Date approved/initiated September 2008	II	N/A	Sweden
	NCT00229736 ^A US-593 ^B	AAV Serotype 2	Aromatic L-Amino Acid Decarboxylase	In progress, Not recruiting - July, 2009	I	Genzyme	USA
Batten Disease, Leukodystrophy, Late Infantile Neuronal Lipofuscinosis	NCT00195143 ^A , US-469 ^B	AAV Serotype 2	Glutamic Acid Decarboxylase 65-67	Completed March 2008 (179,180)	I	Neurologix, Inc.	USA
	NCT00643890 ^A US-877 ^B	AAV Serotype 2	Glutamic Acid Decarboxylase 65-67	In Progress, Actively recruiting - July 2009 ^D	II	Neurologix, Inc.	USA
	FR-041 ^B	Lentivirus	Tyrosinase, GTP cyclohydrolase 1, Dopa-Decarboxylase	In Progress, not recruiting - September 2009 ^D	I/II	Oxford Biomedica	France
	NCT00151216 ^A US-619 ^B	AAV Serotype 2	Tripeptidyl Peptidase (CLN2)	In Progress, Actively recruiting - May 2009 [*]	I	Weill Medical College of Cornell University	USA
	US-381 ^B	AAV Serotype 2	aspartocyclase (ASPA)	Complete - September 2009, Not yet reported		Robert Wood Johnson Medical School	USA

Condition	Identifier ^{A,B}	Viral Vector	Gene(s)	Status ^C	Phase	Primary Sponsor	Location
Leber Congenital Amaurosis, Retinal degeneration	NCT00481546 ^A US-740 ^B	AAV Serotype 2	RPE65	In Progress, Actively recruiting - May 2009 [*]	I	University of Pennsylvania	USA
	NCT00516477 ^A US-677 ^B	AAV Serotype 2	RPE65	In Progress, Actively recruiting -September 2009 [*]	I	Children's Hospital of Philadelphia	USA
	NCT00643747 ^A UK-141 ^B	AAV Serotype 2	RPE65	In Progress, Actively recruiting -February 2009 [*]	I/II	University College, London	UK
	NCT00749957 ^A US-918 ^B	AAV Serotype 2	RPE65	In Progress, Actively recruiting - May 2009 [*]	I/II	Applied Genetic Technologies Corp	USA
	NCT00821340 ^A	AAV Serotype 2	RPE65	Not yet recruiting - January 2009 [*]	I	Hadassah Medical Organization	Israel

^A Identifier for clinicaltrials.gov

^B Identifier for www.wiley.co.uk/genmed/clinical/, last 3 numbers are OBA protocol number for Genetic Modification Clinical Research Information system (GeMCRIS) www.gemcris.od.nih.gov

^C Date of status based on most recent update of three databases (Clinicaltrials.gov, GeMCRIS, Journal of Gene Medicine Clinical Trial Site)

^D See sponsor website for press releases

^{*} See trial database sites for additional information and references

Studies using NTFs shown in bold

N/A = not available

Table 2

Commonly used viral vectors and their properties

	<u>Adeno-associated virus</u>	<u>Adenovirus</u>	<u>Herpes Virus</u>	<u>Lentivirus</u>
Transgene capacity	2–6 kb	8 kb-30 kb	40 kb-150 kb	6–9 kb (proviral length)
Genome	ss DNA	ds DNA	ds DNA	ss RNA
Vector Genome	Episomal,concatamer	Episomal	Episomal, concatamer	Random integration, episomal ¹
CNS Tropism	Neurons and glia	Glia > Neurons	Neurons	Neurons and glia
Expression onset	Days-Weeks	Days	Days	Week
Expression duration	Months - Years	Weeks - Year	Weeks - Year	Months-Years

¹Non-integrating lentiviral vectors have mutant integrase; vector genome is linear or circular DNA episome